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Italian Biotechnology Projects, Research

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National Research Council

93WS0289A Brescia BIOTEC in English Nov-Dec 92 pp 20-22

[Article by Antonio De Flora, director of Target Project Biotechnology and Bioinstrumentation, CNR: "CNR Target Project on Biotechnology and Bioinstrumentation"]

[Text] In 1985, the National Committee on Biotechnology, appointed by the Minister of Scientific and Technological Research and including representatives from academy and industry, was asked to make proposals to foster advanced biotechnology in Italy. The committee, chaired by Professor A. Falaschi, suggested a number of initiatives to be started immediately with a total estimated cost of 1,000 billion Italian lire (1986 currency), to be carried over five years. Besides proposals concerning education and administrative and fiscal facilities for industries (both identified as serious bottlenecks to the development of biotechnology in Italy), it was agreed that launching of specific research programs should no longer be delayed. Accordingly, two main types of programs were suggested by the Committee: one, to be managed by CNR [National Research Council], that included three different Target Projects covering biomedicine, chemistry and agriculture, and had the main goal of strengthening the national network of researchers involved in biotechnology. The second program was identified as a "National Program" on biotechnology, headed by the Ministry of Scientific and Technological Research (MRST), whose aim was the support to industry for pre-competitive research.

Both types of programs were judged acceptable and worthy of funding. However, there was an immediate shortage of funds for both general initiatives compared to the committee's proposals. Specifically, a "National Program" was launched by the MRST (now MURST), with a budget of 209 billion lire, compared to the 400 billion suggested. As to the CNR Target Projects; it was decided that the CNR budget would not allow the immediate start of three different programs. Therefore, a detailed feasibility study (also supported by a "Strategic Project on Biotechnology" headed by Professor E. Cernia) indicated biomedicine and, to a lesser extent, chemistry, as the major areas of applications of biotechnology being most mature for an immediate start. In fact, other CNR Target Projects partially related to biotechnology were funded more recently. In particular "Genetic Engineering"—largely related to basic molecular biology and partially susceptible to biotechnological applications—while "Fine Chemistry 2," "Advanced Research for Innovations in Agriculture" and "Clinical Oncology" are related to biotechnology more marginally through specific technological strategies. The CNR Target Project "Biotechnology and Bioinstrumentation" (BTBS) was eventually approved in 1987 by the CIPE [Interministry Committee for the Economical Programming] and assigned a total budget of 84 billion lire over a five-year period. Based on the feasibility study that had specified the research areas to be covered by the Project, the call for proposals was opened. At the December 1987 deadline, approximately 900 research proposals had been submitted to CNR. The process of selection involved approximately 150 reviewers (a few of whom were from other countries) whose opinions were examined by the Direction and the Scientific Board. Following the process of selection, some research proposals were renegotiated with the proposers and either funded to a limited extent or combined with other proposals. Effective since 1989, the Target Project on Biotechnology and Bioinstrumentation began its activity, which was institutionally monitored every year by the Direction and the Scientific Board. At the end of the first three-year period, in September 1991, a plenary meeting was held in Genoa. This meeting had an attendance of 700 researchers involved in the Target Project, with the display of 400 posters and seven main sessions based on the system of Rapporteurs, i.e., experts in the main topics of the Project reporting on the scientific activity and results of all teams. It was mostly on the basis of the outcome of the Genoa meeting that, at the end of 1991, the Scientific Board decided on the program for the last two years of the Target Project, which should end December 1993. While the activity of 1992 is almost over, a further monitoring is underway in order to detail the program for the last year, which will depend on the still unknown budget to be made available for 1993.

As a research program on biotechnology mostly devoted to healthcare, this Target Project develops its activity along two major trends:

- It pursues the production, by biotechnological approaches, of tools for the diagnosis (e.g., monoclonal antibodies, oligonucleotide probes and related procedures), the prevention (innovative vaccines) and the therapy (drugs) of human and animal diseases.
- It aims at developing new strategies of combined *in vitro* and *in vivo* therapies, based on selected *in vitro* manipulations of cells, tissues and autologous biological material to be then returned to the organism in order to exploit new therapeutic performances.

In order to pursue these two main trends properly, the Target Project involves highly complementary skills not only from researchers in biological and medical branches but also from other disciplines such as chemistry and process engineering.

The scientific organization of the Target Project "Biotechnology and Bioinstrumentation" is shown in Table 1. This reports the current (1992) funding for research and the fellowships allocated to the present 211 teams. Each of the seven Sub-Projects mentioned is coordinated by a responsible scientist (coordinator), mostly through "ad hoc" meetings, seminars, practical courses, assistance to the individual teams in writing patents, etc. The size of the seven Sub-Projects is not uniform in terms of financial resources, this reflecting the grouping ject [as

published] of the different topics defined by the call for proposals under each Sub-Project. The average funding for research (equipment, consumables, travel, fellowships and salaries are excluded) is at present about 58 million lire yearly per team.

Table 1. Organization of the CNR Target Project Biotechnology and Bioinstrumentation

Sub-Project	Coordinator	Teams (No.)	Budget (million lire)	Fellowships (1992)
1. Molecular and cellular engineering	G. Rotilio	43	2.385	21
2. Biodiagnostic tools and innovative vaccines	G. Satta	45	2.463	10
3. Biotransformation and improvement of fermentation processes	E. Cernia	22	1.137	8
4. Biosensors, carriers and cellular bioreactors	G. Milanesi	14	960	11
5. Biotechnological applications to cell cultures and organ transplants	C. Casciani	29	1509	17
6. Bio-drugs	G. Salvatore	30	1676	23
7. Bioinstrumentation	M. Luzzana	28	2174	18

Out of the total budget allotted to research, 59 percent goes to universities, 27 percent to CNR institutions and 14 percent to other research centers being either public or private institutions including industries. The low percentage devoted to industrial research can be explained by the quantitatively higher resources made available to industries through programs of pre-competitive research (like the National Program "Advanced Biotechnologies" coordinated by MURST).

The Target Project "Biotechnology and Bioinstrumentation" is pursuing the following two main objectives:

1) To develop a "critical mass" of researchers in Italy involved in advanced biotechnology.

2) To promote institutional links of cooperation between academic and industrial research.

The first objective is being pursued through two different initiatives. A major one is the support and organization of biotechnological research. This takes place through conventional ways of coordination (meetings, courses, logistic support to researchers, identification and implementation of a few research teams acting as "references" for specific technologies to be spread to other groups) and also by means of flexible interactions with the leading CNR Committee, in order to provide complementary tools of support to the research. The second way was the starting decision to devote a significant, 12 to 13 percent part of the budget to fellowships for graduate students, to be spent in the laboratories of teams enrolled in the Target Project. These fellowships are economically competitive with other temporary positions, and their high number (from 108 to 118 for each of the four years elapsed) is having a positive role on the spreading of those skills and technologies that can be found in several research groups in Italy. Although not yet concluded, the Target Project seems to be in the process of attaining this first objective, i.e., to strengthen biotechnology in Italy by implementing and stimulating the network of institutions active in the area. This conclusion emerges from some of the results obtained, that include: sponsorship (both financial and scientific) of 18 international meetings attended by the best researchers worldwide in topics of interest to the Target Project; over 2,000 papers bearing the acknowledgment to the Target Project and appearance on international journals with referees, besides 1,000 between chapters of books with international editors and papers on national journals; 32 patents deposited or in the process of being deposited. These "measurable" results are paralleled by other certainly not less important outcomes, such as: international collaboration where researchers of this Target Project made it possible to reach fundamental results (a relevant example concerns the role of Claudio Bordignon and his team participating in the first successful experiments of gene therapy in humans); enabling several researchers to participate in specific programs devoted to selected topics, mostly of biomedical interest. These include the BRIDGE program from EEC, programs like AIDS and the Italian Association for Cancer Research, national research programs under the MURST and other Target Projects of CNR requiring sound documentation of knowledge and skill in biotechnology, the activity of the Interuniversity Consortium on Biotechnology, a network joining 16 Italian universities and supporting biotechnological research.

Additional issues demonstrating the vitality of the Target Project concern some international initiatives of public research. Thus, apart from formal presentation of activity at meetings organized by the Ministry of Foreign Affairs (Madrid, 1989; Paris, 1990) or by scientific societies and organizations (Athens, 1988; Milan, 1988 and 1989; Lisbon, 1989; Cleveland, 1989), the Target Project on BTBS has been acting as the Italian counterpart at bilateral meetings between CNR and related

entities like NCRD from Israel ("Immunobiotechnology," Rome, November 1990), KOSEF from Korea (Trieste, April 1992), CSIRO from Australia (Naples, June 1992 and an additional meeting to be held in Melbourne, February 1993). An important initiative concerns the participation of Italy to the European database in biotechnology, called BIOREP (Permanent Inventory of Biotechnology Research Projects in the European Communities, Amsterdam). The Target Project has served and is still serving as the focal point of Italy for this international activity, whereby all biotechnological data of interest are stored in and can be withdrawn from the complete database. To achieve this, all the research teams of the Target Project have been involved.

Finally, the profile of this Target Project, as well as of other related programs and entities involved in biotechnology in Italy, are included in "Biotechnology Worldwide," a general directory published by COBIOTECH in 1991 under the auspices of ICSU (International Council of Scientific Unions). This directory reflects the state-of-the-art in biotechnology in over 50 countries and the national profile of Italy has been prepared jointly by the Director of the Target Project on "Biotechnology and Bioinstrumentation" and by Professor C. Spalla on behalf of ASSOBIOTEC.

As outlined above, the second mission of this Target Project is to create better interactions between academy and industries in the area of biotechnology. This task was much more difficult than the first objective, because these interactions, at least the institutional ones were very poor in Italy for historical reasons and additionally, several constraints are still present that are almost completely unrelated to possible initiatives from both parts. Some of these are dearth of regulations, lack of stable researchers working in biotechnology, variable attitude toward intellectual property rights now aggravated because of current economical problems, growing difficulties in public perception, unsatisfactory or insufficient support to both educational programs and to public research and development programs in industries.

A general and preliminary way to tackle this problem is to provide the two systems of academy and industry with mutual information. With this in mind, in 1989 the Direction of the Target Project and ASSOBIOTEC started a collaboration providing continuous updates of scientific activities to several Italian companies. Under this agreement, in December 1989, a volume was published jointly whose title, "The CNR Target Project 'Biotechnology and Bioinstrumentation': A Bridge Between Academic and Industrial Researchers," is self-explanatory, and which was mostly intended to diffuse information among biotechnological companies. Another volume has recently appeared (July 1992), reporting on the first results of the Target Project susceptible to be transferred to industrial production.

An institutional way to strengthen links between academy and industry was the activation by CNR of the Board of Users (effective 1990), that includes representatives from companies and from other related institutions in Italy interested in the development of biotechnology. The role of this Board in the Target Project is to allow the productive system to express advice, suggestions and criticism on the scientific activity and particularly to identify the outcomes of this activity that may be directly transferred to production. In fact, as repeatedly emerged by a questionnaire circulated among scientists at the plenary Meeting of the Target Project (Genoa, September 1991), the problem of transfer of results from academy to industry is a critical one; a serious bottleneck to the development of biotechnology in Italy being represented by the virtual lack of technology transfer societies.

Since activation of the Target Project, some positive trends have emerged from national companies. The improvement of scientific culture has been witnessed by a considerable increase in the qualitative level of research proposals submitted by Italian companies to the EUREKA program, as repeatedly checked over the last years by the Committee on International Affairs at MURST. In conclusion, it seems that the Target Project 'Biotechnology and Bioinstrumentation' is attaining at least one of the two major preliminary goals, i.e., fostering biotechnological research mostly at universities CNR and other state-funded and private institutions. The second target is currently being pursued with much greater difficulties in view of the still poor gearing between basic and applied research in Italy. The time now seems right for a more specific research program to be built up according to established skills now clearly identified on one hand and to production-oriented topics on the other. Proper choice of trends and topics at the stage of a feasibility study also appears to be possible as a result of careful scrutiny being made by the present Target Project.

Agro-Industrial Productivity

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[Article by Giorgio Ancora, ENEA, Area Innovazione Dipartimento Ricerca e Sviluppo Agroindustriali: "Biotechnology in Plant Production and Plant Protection at ENEA"]

[Text] Over the last few years the R&D activities in Agriculture at ENEA have been influenced by the consideration that in the European Economic Community the increase of the agriculture production has proved, alone, to be insufficient for an equilibrated development of the agroindustrial system which, on the contrary, needs new, advanced and sometimes more complex solutions. Consequently research activities have been oriented taking into consideration: a) the importance of the quality in all agroindustrial products; and b) the

necessity of emphasizing and implementing those technological opportunities capable of minimizing the negative impact of the agriculture activities on the agroecosystem.

Agroecosystem Protection

In the field of agroecosystem protection there are a large number of areas where biotechnology can contribute to solve the number of existing problems.

The ENEA activities in this field are mainly concerned with integrated pest control, aimed at utilizing all suitable techniques and methods for maintaining the pest population at levels below those causing economic injury, reducing costs and harmful effects on sound ecology. In particular, a pilot program of integrated pest control is operating on a large area of olive cultivation (in the area of Canino).

A second approach in plant protection is the biological control, conservation and increment of natural enemies. The main objective of the ENEA activities is the development of strategies and techniques for controlling pests with natural tools. This is pursued through: the application of the sterile insect technique for controlling pests in vast areas (e.g., *Medfly Ceratitis capitata*) or in the greenhouse (e.g., *White fly Trialeurodes vaporariorum*); the construction of biofactories for mass rearing of natural enemies; the design and development of techniques and facilities for mass rearing; transportation and release of useful insects and organisms; the development of programs for the use of biological control in greenhouse in different regions of Italy.

Another line of activity for plant protection deals with the research and development of behavior modifying compounds (sexual pheromones, deterrents, attractants used to monitor and survey pest population and mass trapping).

Plant Breeding and Plant Genetics

Plant breeding and plant genetics is another field in which ENEA has a long experience, also testified by the high number of plant varieties released in cereals, fruit plants, horticultural crops, grain legumes.

The diffusion of some of these varieties (as for instance the cv. Creso of durum wheat) has given rise to an important increase of production, which has resulted in a significant benefit for the farmers.

Of late, the programs of plant genetics and plant breeding have been oriented toward the development of disease resistant varieties aimed at a substantial reduction of chemical pesticides in agriculture.

Among the species considered, beside those mentioned above, there are also some species for industrial and energetic use, like sweet Sorghum, kenaf, rapeseed, sweet potato, jojoba, topinambur.

The plant breeding programs have frequently taken advantage of the application of advanced biotechnologies. In fact when ENEA started in the sixties, it was among the first in Italy to develop research activities in the field of plant cell and tissue cultures.

The development and application of these technologies have been of fundamental importance for reaching the goals of some plant breeding programs. Some examples are: the transfer of useful characters into the cultivated tomato from wild species, thanks to the development of techniques of *in vitro* embryo rescue; the development of primary triticales which has been increased through the embryo rescue after the inter-generic crosses; the isolation of useful mutants after *in vitro* mutation breeding experiments in potato and tomato.

In the potato two new varieties have been released which have been obtained from mutation breeding experiments where gamma radiations have been applied to *in vitro* grown plantlets. Using the same system, experiments of *in vitro* selection are also in progress aimed at isolating potato strains resistant to Phythophtora infestant. Through *in vitro* culture, efficient methodologies of propagation have also been developed, especially in fruit plants and some vegetables. The development of a method of micropropagation for the globe artichoke (Cynara scolymus L), an important vegetable for Italy, is an example of a positive impact of an innovative technology in crop cultivation.

One of the problems of this plant is the difficulty of propagation using offshoots. In fact, after planting, about 50 percent do not root and therefore die, this makes the replacement of the dead plants necessary. The consequences are increased costs and a substantial dishomogeneity of the canopy. In addition, according to this method of propagation in some varieties of high value, like the cv. Romanesco, normally the production of marketable artichokes is expected only in the second year after planting.

According to the procedure developed at ENEA, by using *in vitro* propagated plants practically 100 percent of plantlets transferred into the field survive.

The plants are genetically uniform and pathogen free and, if planted in September, they grow rapidly giving rise in the next spring to a production quantitatively similar to that given by two-year-old plants. This procedure is increasingly utilized by farmers who compensate for the higher costs of plant material with earlier and better production.

Plant Genetic Engineering

Plant genetic engineering is a relatively new field of research at ENEA. It has, however, already given important scientific results from which interesting applications are expected.

Efficient methods of genetic transformation, based on the *Agrobacterium tumefaciens* technology have already

been developed in potato and in *Nicotiana* species and attempts are also being made to transform the globe artichoke and *Pinus* species.

Flow-cytometry is a cell-based technique which enables a fast characterization of a continuous flowing of plant elements on the basis of light emission (fluorescence) and light diffraction (scatter).

This technology is utilized at ENEA for cell characterization, protoplast fusion and for the analysis and sorting of metaphase chromosomes; an approach which promises to become a powerful research tool in plant genetics.

The characterization, cloning and transfer of useful genes are important objectives of the research programs.

Particular attention is devoted to the development of plants resistant to diseases and, in particular, to viral diseases.

The Artichoke Mottled Crinkled Virus (AMCV), a virus whose genome has been completely sequenced and identified in the ENEA laboratories, has been chosen as a model system. Via a multidisciplinary approach (molecular, cellular and genetic), transgenic plants have been created in order to define the mode of action of the virus and the most effective strategy to challenge infection.

In addition, a novel approach is being utilized against plant diseases, which, if the results confirm the promises, could give new opportunities for plant protection. The aim is the transfer of murine genes coding for immunoglobulins raised against plant pathogens. At ENEA the strategy utilized is based on the transfer of genes coding for "single domain antibodies" (polypeptides constituted by VH domains only) which are often able to bind the antigen with good affinity and represent a versatile alternative to the whole antibody molecule.

Transgenic plants have already been obtained which produce high levels of correctly processed "single domain antibodies" (dAbs), while the project for transferring genes coding for immunoglobulins raised against the virus AMCV is in an advanced stage of development.

Soil Biotechnology

In the field of soil biotechnology the ENEA programs are mainly centered on the study of the rhyzosferic microflora and, in particular, on the interactions between some plants and *Azospirillum* and *Rhizobium* bacteria.

Two plants, Sorghum and chick pea, are utilized to study the interaction between *Azospirillum* and plants. Sorghum, which is a good representative of the interaction Azospirillum/grasses has shown a positive response to the infection, while chick pea, a species of increasing interest for Italy, is utilized to study the effect of the interaction between two different nitrogen fixing bacteria on plant growth.

Studies on the competition between different *Rhizobium* strains have also been carried out using *Cicer arietinum.* These studies are aimed at evaluating the competition between different strains of *Rhyzobium leguminosarum* which nodulate the chick pea, when utilized in fields where indigenous bacteria are already present.

Another research in line in this field aims at evaluating the effect of some bacteria (*Acetobacter diazotrophicus* and *Herbaspirillum seropedicae*)—isolated in Brazil from certain sugarcane varieties cultivated in the absence of any mineral nutrition—on the growth of sugar beet and sweet Sorghum, in which these endophytic nitrogen-fixing bacteria were introduced before.

In conclusion, ENEA's activities in plant biotechnology cover several aspects related to plant productivity with respect to the environment and consumers health. In addition, they are characterized by a multidisciplinary approach made possible by the presence of different expertise fields at ENEA (geneticists, biologists, molecular biologists, physiologists, entomologists, chemists).

Science Parks

93WS0289C Brescia BIOTEC in English Nov-Dec 92 pp 31-33

[Article by Domenico Romeo, chairman of the Association of Italian Science and Technology Parks, chairman of the AREA Science Park of Trieste, vice director of the Interuniversity Consortium for Biotechnologies: "Biotechnology Research and Development in Science and Business Parks"]

[Text] In the course of history, man has always made changes to the natural world he finds himself immersed in; manipulating here and there in an effort to render it more to his liking and compliable to his needs, thereby "improving the quality of his life." The means to achieve this end, the entire set of techniques he uses in his labors, is what we call technology.

From the beginning of the last century, technology has been applied mainly as a force to propel the industrial world. The initial impetus, rippling out from Europe, came from the exploitation of coal.

Hot on its footsteps came a phase of innovation based on the arrival of oil, chemical products, and the combustion engine.

We can now consider ourselves to be in the third phase: that of advanced microelectronics, new materials, informatics, telematics, and biotechnologies. In this final decade of the 20th century, technology is playing a decisive role in terms of competitiveness, validity of new products, emerging markets, productivity and profitability of industry, revolutionizing both established and high-technology products, bringing dramatic consequences to the market structure, and taking us into the next millennium on the crest of a fast-moving wave.

In this context, those who stand to succeed will be those entrepreneurs, who know how to identify and exploit the opportunities offered by these new technologies.

One of the leading members of this elite group of high technologies is biotechnology, a combination of techniques and processes that brings together a string of disciplines (such as cellular and molecular biology, chemistry, physics, genetics, immunology, etc.), at the same time fusing together other areas in technology, such as electronics, informatics, and new materials. It is because of this *modus operandi* that biotechnology research centers and companies tend to gravitate towards science and technology research centers and companies tend to gravitate towards science and technology parks, finding there the most fertile environment for success. In addition, given the substantial need for new ventures in biotechnologies, for assistance in administration, financing, and patenting in the early years of their life, they find themselves in a stronger and less vulnerable position if safeguarded within a commercial incubator or park.

Science, research, and technology parks are normally set up near a university or other science centers of excellence, in order to develop applied research projects, starting off from basic research results. The final goal is innovation, in particular in the various branches of high technology, transfering know-how gained in the laboratory to businesses and companies already established at the park or else to new activities attracted by the existence of the park itself. To meet the needs of new ventures like these, innovation centers, incubators or commercial parks can be created with the express aim of giving assistance to fledglings in the delicate phase of their birth and initial development by carrying out innovation and commercialization of products and technological processing with additional assistance in financial areas such as franchising and venture capital. The location of both types of parks within the same compound, constitutes a "technopole." The "technopoles" take on a fundamental role in the building up of an entrepreneurial atmosphere, one that thrashes out new ideas, brings about technology transfer, seeds new activities in economy, and forges international market space for competitive products.

At present, there are over 200 science and technology parks operating worldwide, around half of them in the United States; this figure rises to over 400 if we include those parks being set up or else in the planning phase. In addition, although exact statistics are not available, there are probably hundreds of organizations exclusively devoted to incubation of new enterprises. In our country too, various activities have been launched. The first science park to be created was the Trieste AREA for research in science and technology, operative since 1982. Following on, the Tecnopolis Novus Ortus park at Valenzano near Bari was opened in 1984. Both initiatives, commanding considerable support of public funds, have been promoted by groups of scientists; in the first case working at the university and at the UNESCO/IAEA-run International Center for Theoretical Physics and in the second involving associates from the CSATA (Center for Studies and Applications in Advanced Technologies), a university venture. Later on, an initiative of the IRI (Industrial Public Holding) and of the CNR (National Research Council) led to the creation of the City Consortia for Research, among which there has been a highly successful project in operation since 1986, namely a center for innovation in Genoa, *Genova Ricerche.*

There have also been initiatives launched by organizations of private entrepreneurs. The Bicocca center for technology is being set up in Milan by Pirelli, while at Piacenza, six firms in the mechanics field (Mandelli, Celaschi, Irico, Jobs, MCM, Schiavi), united under the umbrella company 3P—Promotion Pole of Piacenza—are responsible for the launch of the Leonardia center. In Turin, the former car manufacturing plant Lingotto is being converted into a center for innovation, and an incubator for new businesses, as well as a development site for university activity.

On a more recent scale there has been considerable interest in a program launched by three Italian ministries—the Ministry of Budget, the Ministry for Research (i.e., the Ministry for University and Scientific and Technological Research) and the Ministry for the Mezzogiorno (Italy's south).

This involves the creation of a number of science and technology parks in the southern regions of Italy. To comply with this program, a variety of plans for financing have been presented by promotional bodies set up at universities, chambers of commerce, industrial associations, etc. In the feasibility studies of the proposals, aspects of both the entrepreneurial world and of local institutions will have great significance. On the one hand there is a central role to be played by the entrepreneurs prepared to take risks, bringing financing or some of their activities to the park itself, and on the other hand there is the very clear involvement of local institutions in what they can offer in terms of overcoming the barriers arising in the areas of administration, town planning, and infrastructure.

Commercial parks or business incubators have in general the backing of public holdings, both for promoting industrially underdeveloped areas and for finding alternatives to the crisis of industries heading towards obsolescence: in some cases their plans are taken right through to the establishment phase. AGENI, with a vast experience of reconversion in the productive sector of ENI (Public Oil Company), is at the moment engaged in the working out and launching of all-embracing projects concerning the reconversion and development of Italy's south, partly through CTIIS [Technology Centers for Business, Innovation, and Development]. The most prominent projects concern boosting industry and employment in the Val Basento in the Basilicata Region, and in the mining areas of Sardinia, as well as the planning of the Innovation Park Project at Salerno. In a similar way, SPI (IRI's business promotion agency) is carrying out a program to promote centers of industrial development (CISI) and of business innovation (BICs),

some of which, for example the one in Trieste, are already demonstrating outstanding results.

Finally, for both science and business incubators, there is a wide range of projects linked through initiatives of the European Community, such as the SPRINT program, that could find ways to get off the ground in research and innovation centers within the next few years.

All these widespread and diverse initiatives, though, need coordination in order to secure the maximum advantage and efficiency. A network with just this function is offered through the newly established APSTI (Association of Italian Science and Technology Parks), whose goal is to act as an institutional reference point for promoting, evaluating, and developing centers of technology, advising public administrators and investors in planning new initiatives in the area, creating managers to handle these ventures, partly through its connection to similar top international organizations such as the International Association of Science Parks.

As for the activities in research, development, and production in biotechnology, some interesting provisions are made at the Technology Center Bicocca in Milan, which is to offer Milan University's new degree course in biotechnologies. At the Mesagne technology park, near Brindisi, various centers for study and production of biomaterials are planned. The AREA Science Park of Trieste is an interesting case: having chosen to invest effort in the world of biotechnologies, it made a bid for the International Center for Genetic Engineering and Biotechnology in 1983. This center, operated by UNIDO, has been active since the end of 1987 and plays a leading national and international role in biotechnologies applied in the health sphere.

A key feature of science parks is the transfer of technology. It was in the AREA's laboratories that a highly original, rapid, and efficient means of extracting DNA from biomaterials was conceived: the result of this basic research has been a new business, created *ad hoc* and in operation at the BIC Trieste. This is a perfect example of the symbiotic functioning of a science park and a business incubator. This enterprise, Talent, produces reagent kits and bioinstrumentation, meeting with outstanding success on the international market. In the context of the successful alliance between the AREA Science Park and BIC Trieste, the activity of Vectorpharma International also has a place. This enterprise, involved in the development and production of drug delivery and controlled release systems, developed under BIC, is now enjoying long lists of orders already placed for research and production for national and transnational pharmaceutical companies.

There is a further sector of biotechnologies particularly efficient in a science park that has seen the growth of the university/industry relationship. That is biopolymers derived from renewable resources (animal tissues, macroalgae, bacteria). Staff from the University of Trieste have set up the research centre POLY-bios, launching a laboratory, LBT—Laboratory for Technological Biopolymers, that has become the nation's leading laboratory in polysaccharide research and development.

LBT has been carrying out various aspects of research for Italian chemical and pharmaceutical industries, and is very active in the National Research Programs in Advanced Biotechnologies and Innovative Materials, as well as in projects under the European Community's Mediterranean Programs.

This laboratory has already been working on the characterization of a variety of pharmaceutical market products and innovative biomaterial used in health care.

One of the functions of a science park is also to act as a node in the national and international research network. In 1987, the University of Trieste activated the initiative to set up a CIB—Interuniversity Consortium for Biotechnologies, that went into operation in 1989. The Consortium now counts another 15 universities among its members (Bari, Bologna, Brescia, Catania, Ferrara, Florence, Genoa, Milan, Padua, Parma, Rome "La Sapienza," Udine, Urbino, Verona, Viterbo "della Tuscia"). In addition to performing activities at the member universities, CIB has recently launched a national laboratory at the AREA, with research programs on the human genome, and has also started up projects whose goals are to develop new diagnostic methods and bioinstrumentation. In this case too, in line with both the policies and opportunities found at a science park, the CIB laboratory is to carry out research and development activities in collaboration with industries in biotechnology.

A further addition to the AREA technologies, a powerful new research tool will soon become available in the form of the synchrotron light laboratory known as "Elettra." This is a new development at the science park and will be of enormous potential for advanced biotechnologies when it comes on line in 1993. In this laboratory, particularly brilliant X-rays will be produced, bringing an intense monochromatic photon flux to converge on the tiniest samples as a beam 100 to 1,000 times brighter than that of comparable machines now in operation in Europe (Berlin, Daresbury, Orsay), in the United States (Brookhaven, Stanford), and in Japan (Tokyo, Tsukuba).

Another key property of "Elettra" is to be its time structure—the light emission will be pulsed with the accuracy and stability of a quartz clock, allowing top-quality experiments in the kinetics and dynamics of biological systems.

Beamlines for crystallography, optical absorption and fluorescence spectroscopy and microscopy will permit investigations on the structure and function of very large biomolecules, and on the substructure of cells and tissue. In this case, too, in addition to the benefits accruing from basic research, the fall-out in the pharmaceutical and chemical industries, with the possibility of developing new drugs and vaccines, will be very significant.

At present the innovation scenario in our country is giving rise to great excitement which will be directly related to the activities of its science parks and business incubators. The setting up of a network of parks will allow the possibility to address the following:

- the question of qualified staff;
- setting up centers from which technologies can flow;
- creation of a setting conducive to the business world;
- opportunities for triggering new undertakings;
- advanced laboratories for analysis and certification testing;
- advice on innumerable aspects of high technology.

Those who stand to gain are those who represent the competitive power of the country: with an increasing number of science parks and business incubators there will be a growth in the "intangible capital" of the 1990s, defined by the European Community as qualified professional staff, technological skills and know-how and organizational capacity.

Recombinant DNA Plant Research

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[Article by Milvia Luisa Racchi, Istituto Agronomico per l'Oltremare, Florence: "Current Research on Plant Biotechnology in Italy"]

[Text] In the past 10 years the global amount of research on biotechnology has considerably increased, this progress being recognized and supported by universities, state governments and private industries around the world.

This is true for Italy too where the development of research in biotechnology has been greatly supported by the state government in the form of CNR (National Research Council), MURST [Ministry for University Education and Scientific and Technological Research] and MAF [Ministry for Agriculture and Forestry].

Recombinant DNA technology encompasses a vast range of research areas involving basic and applied research in animals, plants and microorganisms and after a period of perhaps exaggerated emphasis and short term expectations, biotechnology is now really coming out from the labs into the fields and factories. Agriculture is a natural area for the applications of biotechnology, considering the fact that the agriculture of the 21st century has to face two different demands, and contrasting the need to feed billions of people who live on this planet and on the other hand, a market in the developed countries, where products have to fulfill the demands of industry and consumer and where quality is more important than quantity. [sentence as published]

Classical breeding achieved great results particularly through the constitution of hybrid varieties; great improvements in yield, quality and quantity and disease resistance were obtained. Now recombinant DNA techniques added to conventional plant breeding open up new strategies either for the production of new varieties more corresponding to industrial demands or to improve the efficiency.

The development of genetic engineering techniques together with the development of *in vitro* culture methods, brought about the technology for the identification and cloning of wide varieties of genes and for introducing these genes into plants. Now theoretically any gene of interest which is present in nature or can be constructed in the laboratories, can be isolated and then inserted and expressed into plants. In addition the genes can be modified to code for proteins with improved enzyme activity or qualitative value.

To date the success of a genetic engineering program depends on the efficiency of identification and isolation of genes conferring traits of economic interest and the efficiency with which a particular species or cultivar can be transformed.

The identification of useful genes undoubtedly remains the single biggest technical limitation in the field of plant biotechnology today. Up to now successful results have involved the manipulation of single gene characteristics. Consequently new genes which confer agronomically important traits to plants need to be identified and isolated. Transposon tagging and chromosome walking potentially represent extremely powerful approaches for cloning new genes. Transposon tagging represents a more direct way to tag a gene. Insertion of a transposable element into a gene causing gene inactivation often gives rise to phenotypical alterations easily detected. The mutated gene is simultaneously tagged, which makes the isolation possible using the transposone as a molecular probe. This technique is used to clone the regulatory genes in the biosynthesis of storage proteins (O7, Fl2, De-b30) and genes with gametophytic expression (de-ga) in maize where transposon tagging has been well established. Efforts to introduce the maize transposons in other important species like tomato, in which transposable elements have not yet been isolated or characterized, are actively underway in order to extend the transposon tagging methodology to a wide number of crops.

Although up to now more than 40 different plant species have been successfully transformed, at present there is not a universal transformation procedure and transformation is still far from being a convenient method; thus the development of routine transformation procedures for a number of crops is a main goal in plant biotechnology. Advances in plant transformation techniques and the development of more sophisticated strategies for enhancing the control of gene expression are fundamental in order to increase the number of crop plants that can be improved using genetic engineering techniques.

Many groups in Italy either in the universities, CNR, MAF or in private institutions have focused their research on transformation methods for many different species. Attention is given to plants of agronomic value like tomato, potato, bean, cereals (maize, rice and wheat) and moreover to poplar. Because of the great value of these crops, the research projects are devoted to the improvements of gene transfer techniques using a wide array of methods, ranging from the use of *Agrobacterium* and transformation of protoplast to the macroinjection of plasmid DNA in immature ovaries of rice, the particle bombardment system into intact tissues and meristems. Genes that confer resistance to antibiotics or herbicides like *bar*, the gene encoding the enzyme phosphinothricin acetyltransferase (PAT) which detoxifies either phosphinothricin or bialaphos, are used as selectable markers for the recovery of transformed cells or plants and because of their agronomic value.

A crucial point in gene transformation experiments is the level of gene expression present in the cells after transformation. Appropriate strong promoters with both a high spatial and temporal specificity, in order to target the gene in a very precise way, are needed, therefore promoter studies are intensely carried out.

The fine characterization at molecular level of the genetic system controlling plant sexual reproduction processes is fundamental for gaining a better understanding of these phenomena, crucial both for the evolutive success of the species, and for the production in crop plants.

Direct manipulation of the reproduction systems requires the isolation of the genes controlling pollen development and function. The possible applications of the outcome of this kind of study is very well exemplified by the production of engineered male sterility plants by triggering "suicidal" genes under the control of appropriate promoters (PGS, Belgium). In Italy several groups are studying the molecular aspects of the genetic control of microsporogenesis, cytoplasmic male sterility and pollen-pistil interaction in plant model systems and in crops. Although the isolation of single genes and their introduction into plants has already provided more than promising results; a "biotechnological green revolution" in agriculture cannot be based on just these approaches. The small number of plant genes cloned so far, the difficulties of transforming plants with more than a few genes, together with the knowledge that most of the agriculturally important traits such as yield, yield stability in limiting or stressful environments, quality of the products, is under the control of very complex genetic systems, therefore limiting the use of engineered plants in crop improvement. Molecular biology can provide alternative tools for studying the genetic basis of complex traits by means of molecular markers. Molecular genetic markers such as RFLPs and RAPDs, able to identify differences at the DNA level, can be used for localizing Quantitative Traits Loci (QTLs) for varietal and species characterization and for evolutionary studies. The approach based on molecular marker analysis has been adopted by many research groups in public and private institutions all around the world. The same is true for Italy where several groups are actually involved in producing genetic maps in crops, such as maize, wheat, barley, sorghum and sugar beet, and in studying QTLs for yield components and for response to biotic and abiotic stresses. Furthermore an important research line is represented by germplasm analysis for Mediterranean species.

Identification and physical mapping of the genes underlying all these characters could be achieved in the near future by means of the most recent advances in DNA technologies such as pulse field gel electrophoresis (CHIEF) and yeast artificial chromosomes (YAC).

One of the goals of plant biotechnology in agriculture is strictly related to crop protection. About one-third of the food production in the world is lost as result of disease, weed competition and predators. This has led to an extensive use of pesticides and herbicides which are extremely toxic and environmentally unsafe. Crop protection has become a "must" and plant biotechnology achieved its first successful applications in this area. Engineering plant resistance to biotic stresses represents the focus of many laboratories around the world and also in Italy several groups are actively involved in the development of new strategies to protect plants from pathogens and of molecular probes for early diagnosis.

Serological techniques may find serious limitations when applied to unstable virus, virus with poor immunogenic properties or in the case of non-encapsidated double stranded or single stranded RNA virus and moreover for the detection of infective agents deprived of coat protein like viroids. Therefore routine analysis for early and handy diagnosis are needed. Recombinant DNA technology has helped to overcome many of the above problems through the application of nucleic acid hybridization methods using cloned molecular probes. PCR techniques can be applied to amplify small specific sequences in field samples and to distinguish very closely related virus strains. Molecular probes based on recombinant DNA technology have been developed for several plant viruses like Grapevine closterovirus A (GVA), Artichoke mottled crinkle tombus virus (AMCV), Artichoke latent potyvirus (ALV), Cucumber mosaic cucumovirus (CNV). A new approach to plant protection is the use of "single domain antibodies" that have been shown to be correctly expressed and post translationally processed in plants. The interference mediated by the "phytoantibodies" expressed as single VH domain represent an entirely new and useful system, in monitoring fundamental processes of plant pathogenesis.

Specific cloned probes have been developed for many plant viruses, like Grapevine closterovirus A (GVA), Artichoke mottled crinkle tombovirus (AMCV),

Cucumber mosaic cucumovirus (CMV). The same technical approach is used to detect the presence of pathogenic fungi or specific bacterial strains directly in the soil.

A new strategy to protect plants from pathogen consist in the expression of "single domain antibodies" (dABs) in transgenic plants. "Phytoantibodies" direct against coat protein or against proteins involved in replication assembly; movement of the virus could represent an alternative system to control viral infections in plants.

Protection of plants against insects is another goal successfully achieved through biotechnology. The isolation and the expression in plant cells of the *Bacillus thuringensis* gene encoding for a toxic protein towards larvae of Lepidoptera (Ecogen Inc. USA) has opened up new perspectives in the fight against insects: to develop plants with built-in mechanisms of protection with little or no intervention by man. Studies are carried out in Italy in order to extend this strategy to other insects such as Coleoptera.

The understanding of plant responses to different environmental stresses such as heat, cold and drought which can affect the normal growth and reproduction of the plants and limit their performance is also being studied. A series of physiological and biochemical studies have led to several acquisitions in this field and progress has been made. Specific stress proteins (both heat and cold proteins) have been identified in cereals, many stress-induced genes have been cloned and characterized and their roles in the stress response are now being clarified.

Plant and other cukariotes produce a specific set of HSPs when tissue temperatures increase above optimum growth temperature. Regulation of HSP expression and characterization at molecular and functional level of the major HSPs: HSP90, HSP70, HSP60 and low molecular weight HSPs are being carried out. Proteins from the former group are believed to function as "molecular chaperones." Different stresses often give rise to similar responses because of the similarities which occur in the physiological changes.

Osmotic stress, for example, can be caused by several different environmental factors including drought, desiccation, salt and cold. As the abscissic acid (ABA) hormone is involved in plant responses to osmotic stress, it is not unrealistic to presume that genes related in these stresses can be regulated by ABA. Identification and mapping of major genes controlling the ABA production in wheat by means of RFLP is one of the research projects being carried out in this field. A similar experimental approach has been used to analyze the genetic control of heat shock protein expression in maize.

The major interest of current research is the further understanding of the molecular mechanisms which regulate the growth and development of plants. Up to now very little information is available concerning regulatory systems controlling development. Several years ago, *Agrobacterium T* DNA genes were shown to encode auxin and cytokinin biosynthetic enzymes. Phytohormones play an important role in a variety of developmental and physiological processes. To investigate the role of hormones in the plant cells growth and differentiation, the auxin and cytokinin synthesizing genes present on T-DNA of *A Tumefaciens* and *Rhizogenes* are being used in transformation experiments. This experimental approach, besides evidencing the different action of the genes coded by the T-DNA of the two bacteria, has been demonstrated useful to study the tuning of the defense response in the pathogenic aggression according the hormone content of the cells.

Bearing in mind the situation of current biotechnological research where a wide array of important areas are actively investigated and developed, an inadequate involvement of research on secondary metabolite production should be noted. Plants are the major source of products used in the specialty chemicals industry (pharmaceutical, flavors, fragrances, pigments, natural sweeteners, antimicrobiotica and pesticides). To date, despite the progress in synthetic organic chemistry, about 25 percent of all pharmaceuticals are directly extracted from plants. Attempts to over-produce secondary metabolites in plant cell cultures have not been very successful so far. Nevertheless the importance of these products for industry makes the development of new strategies leading to a higher production of economically important secondary metabolites in plant cell of great interest. Basic research on the genetic control of the biosynthetic pathway of some secondary products is carried out, but a wide and more coordinated effort in this field is needed in order to achieve relevant results.

Biotechnology for the Mediterranean

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[Article by Manuela Cerimoniale, Society for the Transfer of Biotechnology, Rome: "Biotechnology for the Mediterranean Region"]

[Text] "Mediterranean region countries" stands for countries with considerably different characteristics, especially for what concerns their actual degree of internal resources utilization and their technological system. It includes countries like Italy, Spain, Portugal, and Israel with a relatively high per capita income and advanced industrialization, as well as countries like Algeria, Egypt, Tunisia and Turkey still in the process of consolidating their programming and the kind of development they intend to pursue.

Can a common denominator be possibly identified which, though allowing for wide margins of specific variations, is representative of a trend for all the countries of the above-mentioned region, even if they are both economically and culturally quite different from one another? Such a common denominator might be represented by the need for greater and better utilization of natural resources. In fact, the shortage of fossil and ore

resources in many of the Mediterranean countries, the considerable demographic expansion, even over a short period, in a number of them, and finally their impelling need for substantial improvement of life quality, urge, or should urge towards an increasing capacity for the better use of goods obtained from land cultivation and from the utilization of internal and external waters. Availability of such resources actually represents a remarkably significant component, linked as they are to optimal climatic conditions which more or less distinguish all Mediterranean countries; full play could be given to their utilization through use of the so-called "new biotechnologies."

No single strategy exists, of course, which can be adopted in every situation to take advantage of all the opportunities provided by biotechnologies. These technologies are generally interesting, inasmuch as they provide instruments for development likely to be employed in various fields, such as food production, zootechny, pharmaceutical and chemical industries, biomass cultivation and conversion, transformation and utilization of agricultural by-products, pollution survey and environmental hygiene.

The European Community Commission, through common research programs, already developed a policy in the field of biotechnology aimed at the integration and strengthening of sectorial policies of its member countries which include some of the Mediterranean region countries. Such intention implies a supporting action for the harmonization and improvement of the conditions within which research, development and industrial realization activities are carried out. This policy in fact applies to the entire Community, including the Mediterranean region, in particular through the MIP (Mediterranean Integrated Plans), which should provide a contribution of ECU6.6 million—half of which for agriculture, forestry and fishery—to the Mediterranean regions of Greece, Italy and France.

Among the great opportunities provided to the Mediterranean region biotechnologies, there is the possibility of exploiting a huge quantity of renewable energies.

A UN report estimated that in the western Mediterranean region alone, 156 million acres are uncultivated, though suitable for growing trees and essential products. In order to recuperate the Mediterranean regions for agriculture it is therefore necessary to start on a wide forestation program. According to a recent report worked out by the UN Organization for Food and Agriculture (FAO), environmental destruction, demographic expansion and life-style changes will cause the extinction of 40,000 plant species within the middle of the 21st century, and the world agriculture might even be threatened if protection measures are not immediately stepped up.

Both at national and international level, the strategic trends concerning research and agricultural development appear to aim at this development orientation; towards not merely an increase of the system productivity, but above all towards quality improvement of agricultural production, which, among other things, can be obtained through careful use of the new biotechnologies. In order to prevent the threat of genetic resources erosion, in 1983 FAO set up the Global System for the preservation and utilization of phytogenetic resources, which includes the Intergovernmental Commission and a basic legal structure; the International Agreement on Phytogenetic Resources has been supported by 130 countries up to date.

The problem of erosion of the planet's phytopathogenic diversity arises in this new phase. While primitive man made use of many thousand plant species for his food supply, his modern descendants only cultivate about 150 species, while the great majority of mankind is reduced to utilizing only 12 of them; a restricted high-yield group replacing a wide range of local heterogeneous varieties which therefore risk being lost forever. In Greece, for instance, 95 percent of the original wheat varieties have disappeared in the course of the past 40 years. In Spain, the present Secretary of the Commission on Phytogenetic Resources, Mr. Jose Esquinas-Alcazar, collected more than 300 sample varieties of melon in 1970. Only four years later, three out of 10 specimens were already extinct. The problem is that the loss of local species or varieties means the irreversible loss of biological diversity—known as "genetic erosion." This in turn implies a dangerous reduction of the genetic supplies available for the natural selection process and seriously increases the agricultural crops vulnerability to sudden climatic changes or to the outbreak of new epidemics. Another important fact is that genetic diversity is not uniformly distributed all over the world, being concentrated in the tropical and sub-tropical areas. This is where the greatest number of developing countries, which have no economic resources to protect, manage and classify this common heritage of mankind, are to be found. The following areas have the greatest genetic wealth of edible plants: Mexico, Central America, the Andean countries, the Mediterranean basin, Central Asia, Brazil and Paraguay, the Middle East, Chile, China, Ethiopia, India and the Indo-Malaysian region.

In 1989, all the FAO Member States approved a resolution recognizing the farmers right to existing plant varieties, to crops developed by farmers generations and their rights to new hybrids.

Subsequently, in 1991, FAO's Associate Member States, among which the Mediterranean region countries, approved a new and complementary resolution stating that "the farmers' rights" would be realized through an international fund on phytogenetic resources for the support to programs envisaging preservation and utilization of such resources. Furthermore, within its Global Systems and in collaboration with the International Council on Phytogenetic Resources, FAO is developing a world information and early alert system which might enable quick identification of the dangers threatening basic collection operations and the risk of plant species

and genetic varieties extinction all over the world. Upon request from the Commission of Phytogenetic Resources, FAO is at present preparing two behavioral codes, one relating to collection and transfer of phytogenetic resources, and the other to biotechnology utilization and consequences for plant germoplasm.

Thanks to the application and diffusion in the agricultural sector of advanced technologies such as biotechnology, the opportunity might however arise for the entire world economy, and in particular for the Mediterranean countries, to be part of an extraordinary technological escalation, the market importance and repercussions of which are still unexplored. In fact precisely in this field, and second only to that of human health, biotechnologies are expected to have great impact with the production of improved plant species and the introduction of biological feeding and protection systems which may revolutionize all aspects of the future agricultural world: procedures, yields, efficiency, quality, environmental impact and so on. The most ambitious projects are probably in the field of genetic improvement, some of which have already achieved positive results in producing new plant species, with improved feeding characteristics: increased protein content, improved amino-acid profile etc., plants which can utilize atmospheric nitrogen, plants resistant to biotic stress (insects, pathogens etc.) and to aboisis (drought, salinity etc.).

In addition to traditional plant selection, technologies of agricultural interest, new methods based on cell biology and molecular biology are being implemented nowadays. In suitable conditions, it is not only possible to cultivate plant tissues and cells *in vitro*, but also to obtain their regeneration. *In vitro* propagation of plants, or micropropagation, is a particular technique enabling to obtain, in a short time and in reduced space, a great number of specimens exactly the same as the original plant, starting with cultures of plant cells or tissues. A remarkable advantage of micropropagation is provided by the possibility to obtain healthy plants, exempt from virus diseases. In the last few years, there has been an increasing interest for micropropagation: if we think for a moment that at the beginning the only species reproduced *in vitro* were high-value commercial species such as orchids and ornamental plants in general. Today, methods for *in vitro* propagation of a number of plant species of agronomical interest including grapes (*Vitis vinifera*), potato and citrus are in advanced experimental stages. In plants obtained by micropropagation from somatic cell cultures some mutations occasionally occur (somaclonic variations) which may produce improved genetic characteristics. Micropropagation can therefore be utilized to obtain new improved plant species too. "In Morocco, for instance, *in vitro* micropropagation of numerous agricultural plants species is considered a method for increasing yields and improving the quality of products, so as to better face competition on the international markets of other countries exporting fruits and vegetables, particularly within the European Economic Community. *In vitro* culture was utilized for the introduction of banana cultivation in plastic greenhouses: in a few months this culture, practically non-existent in Morocco, spread out over 500 hectares, utilizing one million small plants" (Sasson).

The modifications obtained by genetic engineering, through the insertion of exogenous genes into the plant DNA, have a set target; it is in fact possible to obtain modified plant species resistant to biotic stress (plants resistant to insects or to viruses, such as the tomato CMV virus), or to abiotic stress (drought, salinity etc.).

The new molecular biology techniques opened new prospects for the realization of new plants not only, but also, for the study of all aspects concerning biological process regulation. They therefore provide a basis for complementing and strengthening rather than replacing traditional methods of new plant species selection. One particularly effective technique for genetic selection of improved species is the RFLP technique (Restriction Fragment Length Polymorphism). Application of the RFLP technique, originally developed in human genetics allows for a more accurate examination of the plant genome composition and for an ascertainment of the presence of a given gene without having to wait for plant regeneration or phenotype expression.

The study of plant nutrition and the role carried out in this connection by some specific micro-organisms enabled to define biotechnological application prospects of great significance in this field. Nitrogen fixation has long been the object of intensive studies to make the biochemical basis process clear, for a larger scale utilization of microbiological systems in the atmospheric nitrogen fixation and a reduced use of chemical manure in agriculture. Inoculations of rhyzobious cultures have been in use for some time already, particularly for soja, while a series of micro-organisms are under study at present (Azospirilli etc.) which provide great application prospects for a number of cultures of agronomic interest.

Other interesting biotechnology applications in plant nutrition concern the utilization of agro-industry residues in fertilizer production. In particular, the biological "composting" process may provide an opportunity to solve environmental protection problems (waste discharge) as well as the supply of organic fertilizers in agriculture. The scientific results obtained from biotechnology in the field of plant protection is also quite promising. The use of bacteria-based "biopesticides," the best known of which is *Bacillus thuringiensis*, specifically allows the destruction of infestants (lepidopters, dipterans etc.) of relevant agronomic species common to the Mediterranean countries, such as grapes and olive-trees. Not only bacteria, but also viruses, fungi, nematodes and other auxiliaries offer biological control with satisfactory results, as an alternative to the traditional chemical action. Some of the bioanalytical methods, long recognized in clinical diagnostics, are extending to the agricultural field. These systems utilize active biological elements (enzymes, antibodies, viruses, nucleic acids etc.) in order to point out the presence of infestant or

infecting phytopathogens. Through the use of nucleic probes it is in fact possible to achieve an extremely high specificity for the determination of microbic and virus infections. Other bioanalytical methods, in particular those of the immunologic or enzymatic type, are becoming increasingly simple to use even "in the field," besides being more specific and accurate. Within the frame of diagnostics, the potential of the new RFLP (Restriction Fragment Length Polymorphism) techniques should be pointed out, as well as those of the PCR (Polymerase Chain Reaction) techniques which can introduce innovations into the traditional cultivar selection techniques and may considerably increase the sensitivity of some tests based on the use of acid nucleic probes, for instance allowing a timely survey of pathogens presence in a crop. Such technologies can be applied to diagnose some of the most serious phytopathologies of plant species typical of the Mediterranean countries, such as grapes, citrus etc. (*Phoma tracheiphila*, citrus disease, etc.).

With regard to the zootechny field, it should be pointed out that in the last decades European countries witnessed the extinction of about half the domestic animal races which existed at the beginning of the century, while one-third of the remaining 770 species are threatened by extinction within the next 20 years. The situation is even more serious in developing countries, as the original animal species are risking extinction under the pressure of imported races competition. The sheep of the island of Chios, in the Aegeus Sea, is the perfect example of a race which might be interesting to study due to the quantity and quality of milk it produces which is particularly resistant to heat. The Fayoumi chicken, originating from Egypt, can produce eggs in high-temperature climatic conditions which would be prohibitive for western species breeding. This species, dating back to the Pharaohs' time, is risking extinction as Egyptian breeders are trying to replace it with western chickens or with cross-bred races. FAO prepared a five-year plan for the protection of animal genetic resources, including the following points:

1) Census of the world animal species.

2) Realization of a seed and frozen animal embryo bank for particularly valuable species threatened by extinction.

3) Preservation programs of species threatened by extinction in their original habitat.

4) Use of DNA technology to determine the genetic characteristics of animal species in developing countries and to assist in programs for the improvement of animal species selection.

5) Development of a first legal scheme at international level for the protection of copy-right (trade marks) and for the safeguarding of animal genetic resources.

The genetic improvement aspect has always been of great significance to breeders interested in propagation of the genetic heredity of specimens with interesting characteristics in the shortest possible time. Artificial insemination, through which propagation of particularly valuable genotypes was carried out, is one of the first techniques that in some way opened the way to biotechnology application in the zootechny field. Propagation of *in vitro* techniques applied to embryo insemination, development, replanting or transfer represents a further method aimed at obtaining a great number of animals deriving from mating of high-genealogy specimens. Furthermore, a remarkable contribution comes from molecular biology techniques for the production of individuals with modified genetic heredity (transgenic animals). Interesting experiences were carried out with regard to the expression of gene coding proteins of pharmaceutical interest, integrated with gene coding the milk proteins of cows, goats and other species, so as to obtain transgenic animals capable of secreting considerable quantities of such exogenous proteins in milk. Particular importance is also attributed to the applications of techniques typical of diagnostics, with the purpose of identifying genotypes or the embryo sex, so as to improve the selection techniques effectiveness. Techniques for obtaining DNA fingerprints useful for paternity tests also appear to be interesting.

For what concerns the nutritional aspects of zootechnic food, there are many biotechnology applications by which it is possible to increase food digestibility, to decrease losses during preservation and at the same time raise the nutritional value of forage during ensilage. In particular, according to the results of such studies, the *Saccaromices cerevisiae* yeast appears to be the most promising due to some characteristics, such as glutamic acid production, which increases food desirability; biomolecules secretion (vitamins, nucleotids, amino-acids, etc.) even in a scarcely favorable-to-development environment and activity on rumen pH. Addition of selected yeast in the feed portion of monogastric animals allows to increase feeding efficiency.

Among the most common veterinary therapeutical products, antibiotics can be considered as some of the first and more widespread biotech products. These are molecules produced by various micro-organisms (fungi, actinomyces and bacteria) and can inhibit the growth of other germs or cause their death. Among the biotech products relating to veterinary prophylaxis, vaccines obtained through recombinant DNA techniques should be mentioned. In fact, proteins (or their fragments often presenting greater antigenic properties) are utilized as "safe" vaccines, lacking the genetic material essential to the development of pathogenic characteristics. For what concerns the diagnosis of viral animal diseases, direct and indirect laboratory tests exist. The former are based on the straight identification of the infectant agent, while the latter utilize the antibody properties of the affected organism or the antigenic characteristics of the pathogen (ELISA and RIA tests).

Preparation of some traditional food through micro-organisms may be chronologically considered as the first

application of biotechnology by man (production of fermented drinks, bread leavening, milk coagulation for cheese-making etc.). An extraordinary bas-relief, discovered inside an Egyptian tomb of the V dynasty (2500 B.C.), and illustrating a sequence of all the different phases of beer production, provides evidence of the forementioned. Nowadays, biotechnology offers new and more powerful instruments for the control of procedures on which many food productions are based.

Since the 1940s, selection of specialized micro-organisms and preparation of starters to be utilized in various fermentative processes, as well as the development of fermentation engineering, provided issues of great interest within the frame of biotechnology applied to food production. The evolution of applied microbiology developed into the two phases of selection of species and of breeds specifically designed for fermented products, and subsequently adopted genetic interventions to obtain requisites which are absent in kin breeds. In the food industry sector, yeasts are of the greatest interest, as both quantitatively and economically they represent the larger group of micro-organisms utilized by man; the world yearly production is estimated around 2 million tons. Important applications concern the fields of wine and beer. Milk enzymes are also very important, as they could be utilized in various agro-alimentary sectors concerning the typical productions of Mediterranean countries: selected starters for vinification, use of milk starters for direct inoculation in the boiler, use of mesophyl milk bacteria selected for milk pre-incubation in order to control the development of undesired psychrophilic flora, improvement of fermented milk (yogurt), lactobacilli as preserving agents in the food industry (bakery products, olives, sausages and seasoned meat, pickles etc.).

Considerable progress was made with regards to interpretation and utilization of enzyme activities constituting the extremely wide range of biological material used in feeding, as well as of the activity of exogenous enzymes employed in transformations for alimentary purposes. Interest for enzyme treatment utilization is such as to have caused a drastic evolution in some production fields. The following can provide some examples of interesting applications for the Mediterranean countries: sterilization methods for the dairy industry; enzyme hydrolysis of lactose for the production of special milks; enzyme mixes to accelerate the ripening of dairy products; pectase for wine and fruit juice clarification; lipolitic enzymes lipase for production of fat acids and new products (dietetic oils); lysozyme as bacteriostatic agent; bakery industry amylase; meat, dairy and bakery industry protease; glucose oxidase for the color and flavoring preservation of foodstuffs; microbial cheese rennet and so on.

Even non-biological technologies, specifically developed for the treatment of biological material, are by now commonly classified as biotechnologies, taking into account their peculiar characteristics. These technologies often concern the separation and/or purification of biological components (downstream processing or bioseparation), or they may concern the selective treatment of certain components of a biological matrix. In particular, this latter kind of technology is very important in food transformation, where the precise aim is to transform certain components without damaging others, that is avoiding "technological damage" connected with harsh treatments; for instance thermic and mechanical damage, contamination etc. Application examples may include membrane procedures for wine sterilization, for the production of low-alcohol wines, for the standardization of the fat/protein percentage in cheese-making milk, etc., fruit and vegetable preservation in controlled atmosphere, flavor extraction by supercritical fluids, essences from officinal plants, etc., new methods of milk cold-treatment (pasteurization/sterilization) employing no chemical additives, new vinification methods through temperature control.

With regard to the food industry, the diagnostics and quality control issues are of basic importance. In recent years a combination of the technological progress together with an increased interest both on the part of consumers and by the bodies responsible for regulations on foodstuffs security, accomplished remarkable achievements in the diagnostic and analytic fields concerning the quality control of agricultural and food products. Bacteria, fungi, molds, microbial toxins and drug residues are among the contaminating agents which cause the most serious worries to food producers and consumers. Traditional tests concerning some of these agents require long and elaborate culture procedures with the samples under examination, and/or complex chromatographic tests; identification of other pathogens through such techniques is very difficult or even impossible. Instead, biotechnology by making use of the extraordinary selectivity and sensitivity properties of biological materials, provides a possibility to have more and precise analyses, as well as to set up simple field kits which can be utilized even by people with no deep analytical knowledge.

A certain number of marine sciences such as algology, marine microbiology, chemistry of sea-organisms' natural compounds, and marine ecologic chemistry, recently discovered the potential of biotechnology thanks to the launching of research programs for the isolation of marine bacteria capable of rapidly consuming oil, for large-scale production of bioactive compounds, structural polymers and foodstuffs of commercial interest, for the industrial employment of bioluminescence, the development of antifouling agents and hydroadhesives. In this connection, exploitation of the huge marine resources, under-utilized at present, would create an encouraging impact. Contrary to agriculture, aquaculture has gone through deep technological changes over the past 100 to 150 years, representing a still rapidly expanding biotechnological field. Applications of biology, molecular genetics and bioinformatics are interesting both for the analysis of marine organisms

biovariety, intended as a genetic wealth resource to be preserved, and for their engineering. For what concerns this latter aspect, a greatly expanding area is represented by genetic manipulation of marine organisms specifically designed for production of higher quality and productive yield breeds, as well as for a better understanding of the genetic expression mechanisms. A number of experiments were carried out on different kinds of fish, through various techniques, such as microinjection of the genes or other complex and delicate methodologies. Suitably treated animals may result in being larger, besides showing a higher resistance to diseases. An attempt was made, for instance, to introduce the anti-freeze protein gene (AFP) into the salmon set of genes; these proteins are fundamental for the survival of fish species in waters reaching even -2°C, as they prevent blood freezing.

Environmental Research

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[Article by Guido Grandi, ENIRICERCHE: "Environment and Research Activities at ENIRICERCHE"]

[Text] Environmental control is no doubt an issue of general concern and this is particularly true for highly populated countries like Italy, where pollution is creating critical situations.

In this context, it is important to concentrate research efforts for the development of efficient systems able to clean up already contaminated environments and develop new and safer products and production processes.

Among the Italian research centers which are devoting a large part of their activities to environmental production, Eniricerche, the centralized research company of ENI, is undoubtedly one of the leading groups.

The company is carrying on a number of research programs which can be grouped into three wide categories:

- identification of contaminated areas with the use of new diagnostic devices;
- new technologies for waste treatment;
- new products to protect the environment.

In the first category it is worth mentioning an important project, financed by ENI and many companies belonging to the ENI group, which is dedicated to the development of innovative systems for the monitoring of atmospheric and water pollutants. In particular, chemical and biological sensors are under investigation for the detection of heavy metals and pesticides. The biological sensors are based on specific proteins characterized by high affinity for specific metals and on antibodies selected for their property to bind toxic compounds.

In waste treatment, two lines of activity are in progress, one dealing with contaminated soil remediation and the other focused on the treatment of waste waters from different origins.

As far as the first line of activity is concerned, in the effort to clean up a large area of sandy soil contaminated with automobile fuel, technologies have been developed for precisely locating and mapping the level and extent of the oil contamination. In addition, *in situ* and on site bioremediation systems have been adopted with satisfactory results, both in terms of percentage of oil removed and of time required for clean up.

In the sector of wastewater treatment, Eniricerche has a multiannual experience in the anaerobic digestion of the liquid wastes derived from the food and agricultural industries. As a matter of fact, many are the plants installed throughout Italy already producing biogas from a variety of different liquid wastes.

More recently the technology has been applied to urban liquid wastes and in this context a pilot plant, characterized by reduced energy consumption, has been designed which makes use of selected consortia of immobilized anaerobic microorganisms. It is also worth mentioning the construction of a pilot plant for the treatment of solid urban wastes. Starting from the organic fraction of such wastes, the plant is able to produce methyl esters through the combination of biological and chemical processes. Anaerobic digestion is in fact utilized to generate organic acids which are subsequently converted into esters by a chemical reaction. The esters can find important applications in the oil industry as clean additives for automobile fuels.

Eniricerche has now two important activities in progress, one addressed to the synthesis of biosurfactants and the other to the study of biological hydrogen production.

Surfactants find a large number of applications in a variety of industrial sectors including those that utilize these molecules for the bioremediation of soils contaminated with toxic hydrocarbons. In this context, biosurfactants appear to be particularly attractive for their biodegradability. In the attempt to construct a biosurfactant overproducer strain, the Genetic Engineering and Microbiology group of Eniricerche is conducting a detailed study at the biochemical and genetic level of the synthesis mechanism of Surfactin. This is a potent biosurfactant produced by a particular strain of *Bacillus subtilis.* Surfactin is a very interesting molecule formed by a fatty acid moiety, which provides the lipophilic property, and by a seven amino acid peptide responsible for its hydrophility. Eniricerche found that the heptapeptide present in Surfactin is synthesized by a large enzymatic complex which is responsible for the recognition and condensation of each amino acid. The entire operon codifying for the enzymatic complex was completely sequenced and characterized. The understanding of the mechanism of synthesis of Surfactin has opened new possibilities not only for the isolation of engineered

overproducer strains (in which the regulatory elements controlling Surfactin synthesis are properly modified), but also for the production, through the alteration at genetic level of the enzymatic complex, of modified versions of Surfactin in which specific structural changes can provide the new molecules with altered chemical-physical properties. Hydrogen is an ideal fuel as water is its combustion product. Therefore, at least in some applications, its use in place of fossil fuels is highly desirable from an environmental point of view. It is well known that biological systems have the ability to evolve hydrogen under particular circumstances. The most interesting systems are those which make use of solar energy to convert either organic compounds (photosynthetic bacteria) or water (cyanobacteria) to hydrogen. The enzyme hydrogenase plays a key role in most of the biological hydrogen production systems. Therefore, a thorough understanding of the regulation of hydrogenase synthesis in different organisms and of its structure and function might be particularly useful for developing biological systems characterized by more efficient hydrogen evolution properties.

Eniricerche's activity is currently focused on the isolation of hydrogenase genes from thermophilic and iperthermophilic bacteria, with the final goal of improving the hydrogen production capacity of photosynthetic bacteria by overexpressing heterologous hydrogenase genes in these bacterial hosts.

This project is partially funded by the Japanese Ministry of Industrial Trade and Industry (MITI) in the context of a vast program on hydrogen production with five Japanese industrial companies. Eniricerche is the only foreign company taking part in the program.

Bioindustry Development

93WS0289G Brescia BIOTEC in English Nov-Dec 92 pp 88-91

[Article by Celestino Spalla, Finbiotec, Milano: "Bioindustry Development: General and Italian Situation"]

[Text] The new bioindustry is the result of the fusion of two important groups of knowledge, that is:

1) the recent breakthrough discoveries of molecular biology and genetics such as the structure of DNA, the genetic code, protein biosynthesis as well as recombinant DNA and monoclonal antibody technologies;

2) the already industrially exploited technologies of microbial fermentation and of separation and purification of the obtained products.

It appears therefore that the new bioindustry is the result of the combination of recent basic discoveries mainly accomplished in universities and of technological expertise and facilities already present in industry. Traditional fermentation and bioconversion processes largely utilized for the production of antibiotics, amino acids, etc. have now reached a phase of economic maturity without forecasts of significant increase or decrease of volume and sales, and are also characterized by an incredible technological immobility. It is therefore better, for the purpose of this discussion, to set them aside and concentrate on the various aspects of the younger and turbulent advanced bioindustry.

It was at the end of the 1970s that scientists and technologists were able to foresee exciting possibilities for creating new products useful in health care, agriculture, food and protection of the environment. The entire bioindustry was established on the basis of these "possibilities" and "promises" which at the time were just that. Heavy investments in human, as well as financial, resources were made (tens of billions of dollars) all over the world in order to set up research projects in the belief that the products and the markets would shortly follow.

The reality however was not so brilliant, and those who did invest heavily in biotechnology, some 10 or 15 years ago, have been sorely tried and a number of them have "thrown in the towel," so to speak, although many more have taken on the challenge. The difficulties have been many: from technical to legal ones, due to the fact that timeframes proved longer than expected and that the cost for the products/services obtained have been in general too high, etc.

The obvious consequence was that biotechnology, after some years of high interest and years of great hopes, lost at least in part its interest which became very low from 1984-1987. And this happened in spite of the fact that some important products had reached the market. In fact, both human insulin and human growth hormone, even if important, were considered as rather obvious technological discoveries and, as such, without a real strategic value.

As a result, attitudes and strategies had to change, and the bioindustry initiatives were heavily restructured in term of objectives, focus, and management. In this way, as will be discussed later, even in the presence of lower investment, they were able to achieve important success in terms of products and of sales.

The countries where the most important achievements have been reached are the U.S. followed by Japan and Europe. Other countries like Canada and Australia are also active. In Italy, as will be seen later, a somewhat contradictory situation exists as, in spite of an extraordinary development of the traditional fermentation industry and the presence of high quality, fundamental research in molecular biology, the inadequacy of infrastructure for technological transfer and the lack of a modern legislation stimulating industry innovation slow down the industrial investment in biotechnology and biotechnology development.

Bioindustry in the World

The bulk of the present industrial activity in biotechnology is being carried out by a relatively large number of companies (Table 1). First of all there are the NBCs,

New Biotechnology Companies, or NBFs, small, specialized biotechnology companies normally endowed with very high R&D capabilities. These companies played and are playing a very important role in the development of bioindustry and their "story" has provided matter for many fascinating publications.

Table 1. Bioindustry Structures (1990)

Country	Number of companies			
	NBC	EC	Total	Employees
USA	1,100	580	1,680	70,000
West Europe	370	390	760	25,000
Japan	100	400	500	12,000
Total	1,570	1,370	2,940	107,000

NBC: New Biotechnology Company. EC: Established Company.
Source: Biotech. Forum Europe, Financial Times Biotechnology, Ernst & Young.

The second player in biotechnology is represented by the ECs (Established Companies): large, already existing companies (pharmaceutical, chemical, agrochemical, etc.) which added a department of biotechnology to their R&D laboratories. Altogether there are nearly 3,000 biotech companies in the world with about 100,000 employees.

An important index of the interest in biotechnology are the expenses in R&D quoted in million dollars for the U.S. (5,000, 1990), Japan (2,000, 1989) and Europe (2,500, 1990). They include the cost of R&D carried out by NBC and EC and the state grants to the same industry. If the entire R&D (industry plus academia) is considered, a total expense of $16 billion can be estimated, which means that roughly 250,000 persons in the world are doing research in biotechnology.

As for the products (Table 2), the first major breakthrough was human insulin, which came onto the market in 1982, followed, three years later, by the human growth hormone. Further successes have followed. Some 20 pharmaceutical products developed by means of biotechnology techniques are now on the market, some of which are real blockbusters. It is important to take note that the largest number of products reached the market in 1990-1991. To these should be added diagnostic products based on monoclonal antibodies, oligonucleotide probes, enzymes, as well as products for agriculture.

Table 2. Main Pharmaceutical Biotechnology Products on the Market

Product	Indication(s)	Year of appr.
Human insulin	Diabetes	1982
Human growth hormone	Human growth hormone deficiency in children	1985
Interferon-alpha 2b	Hairy cell leukemia	1986
Interferon-alpha 2a	Hairy cell leukemia	
Orthoclone OKT3	Kidney transplant rejection	
Hepatitis B vaccine	Hepatitis B prevention	
Tissue plasminogen activator	Acute myocardial infarction	1987
	Pulmonary embolism	
Interferon-alpha 2b	Genital warts	1988
	AIDS-related Kaposi's sarcoma	
Haemophilus B vaccine	Haemophilus influenzae type B prevention	
Erythropoietin alpha	Dialysis anemia	1989
Interleukin 2	Kidney cancer	
CMV Immune globulin	GMV disease associated with kidney transplantation	1990-91
BCG live	CIS of the urinary bladder	
Factor VIII	Hemophilia A	
Factor IX	Hemophilia B	
Gamma interferon	Chronic granulomatous disease	
G-CSF	Adjunct to chemotherapy	
GM-CSF	Bone marrow transplant	
Glucocerebrosidase	Type 1 Gaucher's disease	
Polio vaccine	Polio immunization	
Centoxin	Septic shock	

Other new products are in the pipeline and, while they are not yet earners at present, they are nonetheless of interest inasmuch as they represent future potential for the bioindustry. In the U.S. alone, a total of 121 pharmaceutical products are "in phase" while 12 products in 1989 and 15 in 1990 have been submitted to FDA for approval.

Many of the new products are in the area of colony stimulating and growth factors, lymphokines, monoclonal antibodies, and vaccines.

The speed with which products have come onto the market is remarkable and, considering the number of products in the pipeline, it is expected to further increase in the future. The greater number of products coming onto the market has led to boosted revenues (Table 3). From less than $3 billion in 1989, biotech products sales reached over $7 billion a year later—a 174 percent boost. About half of these earnings were in the U.S., the rest being equally shared between Japan and Europe.

Table 3. Sales of Biotechnology Products (million dollars)

Country	1990	1989	Percent change
USA	4,000	1,300	207
West Europe	1,800	700	157
Japan	1,600	700	128
Total	7,400	2,700	174

Source: Ernst & Young, Financial Times Biotechnology, Biotech Forum Europe.

Bioindustry in Italy

Italian industry is characterized by deep tradition and excellent know-how in fermentation. Since the 1950s, important fermentation plants have been active producing all the commercially important biosynthetic products both for captive and foreign markets. New products and new processes were developed, some of which of great practical interest (Table 4).

Table 4. Main Products and Processes Developed by the Italian Fermentation Industry

Products	Aminosidine, Daunorubicin, Doxorubicin, Nicergoline, Rifampicin, Teicoplanin
Processes	Beta lactam antibiotics, Erythromycin, Ergot alkaloids, Steroids (bioconversion), Vitamin B 12, Immobilized enzymes, Aspartame, p. Hydroxy-phenilglycine, Pennicillin-acilase, Beta galactosidase, Glutamic acid, Phenilalanine

Interest for industrial applications of the advanced biotechnologies in Italy became really evident in the mid-80s. In those years, in fact, two reports emphasizing the strategic importance of biotechnology were published and the relevant actions to be carried out by both Public Administration and industry were suggested. The first report was the result of the work of the National Biotechnological Committee, chaired by the Ministry of Scientific and Technological Research, while the second one was produced by a committee of experts organized by Federchimica (the Federation of the Italian Chemical Industry). A substantial increase in the R&D expenses, an improved personnel training, the creation of structures for technology transfer, and a new legislation favorable to the investment in biotechnology ventures were the main recommendations of the two committees.

At the beginning of the 90s, the situation of biotechnology applications in Italy was thoroughly reviewed in a comprehensive report once again produced by Federchimica through the affiliated Assobiotec (National Biotechnology Association) and edited by the author. The data quoted in the present article largely refer to this report.

The present bioindustry structure and some relevant data are reported in Table 5. The new biotechnology companies in Italy are generally small (80 percent of them have less than 20 employees) and only a few perform really advanced R&D. It is interesting to note that out of 60 NBCs, at least 40 have been partially financed through venture capital or with intervention of the BIC (Business Innovation Center) or regional funds.

Table 5. Bioindustry Structures in Italy (1991)

New Biotechnology Companies	n.	60
Established Companies	n.	75
Total Biotechnology Companies	n.	135
Employees	n.	2,400
R&D expenses	mill $	150
Revenues of Biotech products	mill $	350

Source: Assobiotec, Interviews.

The number of NBCs could certainly be higher and their quality improved if an antiquated legislation on the financing of the innovation had not hampered their development. It is, however, to be said that, during the past year, a more advanced legislation has been approved and, even if it is rather inadequate, some beneficial effects are expected. About half of the biotechnology companies are associated with Assobiotec which was set up in October 1986 within Federchimica, the Italian Federation of the Chemical Industries. Assobiotec combines the role of providing entrepreneurial support to national and EEC biotechnology developments with the role of being the landmark for companies involved in production and marketing of products derived from the application of both traditional and advanced biotechnology.

As for the sales of biotechnology products, more than 50 percent of them come from the health care sector which will maintain its pre-eminency in the foreseeable future. Forecasts of Assobiotec anticipate sales for more than $2,000 million in 1995 and for about $4,000 million at the end of the century, with the health care sector share accounting for around 50 percent, followed by the food sector with a mere 13 percent. It is important to note,

however, that the majority for biotechnology products sold in Italy are imported, only a fraction being produced by the Italian industry.

An important role in the development of biotechnology is that of the research carried out at the expense of the state both in its own structures (Universities, CNR, ENEA, Istituto Superiore di Sanita, Stazioni sperimentali, etc.) or through funding and grants to industries. The last available data on the involvement of the public administration in biotechnology R&D are reported in Table 6 and refer to 1989. There are no reasons to think that the situation has substantially changed in more recent years.

Table 6. Involvement of the Italian Public Administration in Biotechnology R&D (1989)

Employees	University	n.	3,500
	Other research centers	n.	900
	Total	n.	4,400
Expenses	University and other research centers	mill $	190
	Funding of industrial R&D	mill $	50
	Total	mill $	240

Source: Istat, MURST, Assobiotec.

As far as higher education and biotechnology training are concerned, out of a total of 80,000 graduates each year coming from over 60 Italian universities, 30,000 are graduates in scientific and technical biological disciplines (science, medicine, agriculture, Veterinary, pharmaceutical science, nutrition sciences, etc.) and 3,000 are graduates in engineering. As for the "Laurea in Biotecnologia," although foreseen in the near future, it is not presently operative. The post graduate studies are mainly carried out in specialization schools and in Research Doctorate institutions, totalling a few hundred biotechnologists each year.

Among the main institutions fostering scientific based innovation are the science and technology parks. They have been instrumental in the development of the bioindustry in all the countries: in the U.S. there are 25 parks which generate 100 biotechnology companies and in England and France the parks are 42 and 26, respectively hosting 70 and 32 biotechnology companies. So far in Italy there are only three parks hosting a few biotechnology companies. It must be said, however, that a fairly good number of parks are now rising and that the situation might be solved in a short time. As far as cooperation with the developing countries is concerned, the Italian industry has so far been very active. Important processes and plants for the production of antibiotics and other fermentation products have been built up in many countries (India, Algeria, Argentina, Romania, China, etc.). Advanced biotechnology facilities and know-how are also being supplied, e.g., to Indonesia.

Conclusions

The commercial application of biotechnologies in the world is rapidly growing. About 3,000 biotechnological companies with more than 100,000 employees are active. Several tens of new biotechnology products reached the market with total revenues of more than $7 billion while some 100 products are in advanced phase of development. Furthermore, about $10 billion are invested annually in industrial R&D, and more than $6 billion in fundamental R&D totalling around 250,000 biotech researchers in the world. Real blockbuster products, real industrial companies, real sales and a well-defined and real future based on already-existing products make one confident in affirming that biotechnology is now, even with some delay, well and truly launched and is on line for rapid and sustained growth. As far as the Italian situation is concerned, the available data point out, together with several positive factors, some negative ones which have so far hampered the industrial development of biotechnologies. An excellent tradition and know-how in the fermentation industry and the high quality fundamental biological research are the key points of Italian biotechnology. Also worthy of mention is a strong entrepreneurial vocation confirmed by an extraordinarily elevated natality of new enterprises active in a large number of sectors. Significant improvements have also been made in the last two or three years in the training of personnel. The creation of science and technology parks is in progress but at the moment the functioning ones are no more than three or four.

A new fiscal and financial law in favor of innovative companies has been approved which, even if not completely adequate, could be a good starting point. Industry and financial institutions show interest for biotechnology as can be seen from the fairly good number of new and established biotechnology companies. The investment of adequate amounts of resources, however, is hampered by the scarcity of correct incentives. These incentives could be mainly found through the completion and implementation of the program recommended by the first and second reports of the National Biotechnology Committee, specially the improvement of the relationships between Universities and Industry, the stimulus to pursue more applied research and finally the promotion of technology transfer by stimulating both the functioning of technology parks and the different forms of biotechnology financing. A great deal of work has already been done, substantial resources have been invested; a last and definitive effort is now necessary and mandatory and will certainly permit the Italian industry to reach the same excellent level in advanced biotechnology as that already reached in traditional biotechnology and so continue the intense and fruitful relationships already established with the developing countries.

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Industrial Biotechnology Association

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[Article by Alberto Valvassori, executive director, ASSOBIOTEC: "An Industrial Association of Biotechnology"]

[Text] Assobiotec, the Italian Industrial Association for the Development of Biotechnology, was created in 1986.

According to statute, the attention of the Association is focused on:

- regulatory issues affecting production, marketing and use of biotechnology products, as well as the harmonisation of standards;
- fiscal and financial policies aimed at motivating industrial innovation;
- R&D, education and training projects and programs.

The Association's policy mainly deals with: monitoring proposed legislation in the regulatory area; representing Italian bio-industry at national and international level; providing a regular forum for the exchange of information and initiatives of entrepreneurial relevance; taking active part in the reviewing process relative to the rules on industrial property.

Current Activities, Management and Membership

The current activities of the association can be summarized as follows: active cooperation with regulatory authorities, both at national and international level; close interaction with trade organizations dealing with biotechnology; structured contacts with universities, fostering collaboration between academia and industry in research and education; information services on commercial biotechnologies, on patents and on implementation of R&D programs.

Assobiotec is presently governed by a Steering Board of representatives from 22 member companies elected by the 1992 Ordinary Assembly; the president and two vice presidents are appointed by the board. The association is managed daily by an operative staff composed of the executive director, a projects coordinator, an information scientist and office personnel. Assobiotec consists of only one membership category: profit-making concerns directly involved in biotech business in Italy.

On top of a fixed minimum charge, individual annual fees are calculated according to companies' dimensions (based on both the number of employees involved in biotech activities and the overall financial turnover). At present, 60 companies are associated to Assobiotec.

Working Arrangements

The association's Steering Board has created several working groups, which are formed by staff members and company experts. They are: Research & Development (13 members); Regulations (seven members); Fiscal &

Financial (five members); Patents & Industrial Property (seven members); Training & Education (five members); Process Engineering—including standards (six members).

Assobiotec is assisted by a Presidency Advisory Committee formed by 12 academics; the Committee meets three to four times a year and advises the association on science and technology matters.

Forming Biotechnology Policies

In Italy itself, Assobiotec is currently consulted by government departments and Parliamentary Committees on issues dealing with biotech safety and development; several members of the board and/or the staff are serving in national commissions/committees as bio-industry representatives.

At international level, Assobiotec participates and represents the Italian position at OECD (Organisation for Economic Cooperation and Development) (safety in biotechnology), CEN (European Committee for Standardisation) (standards), EEC (European Economic Community) (regulations), ESNBA (European Secretariat of National BioIndustry Association) (industrial policy). ESNBA stems [as published] for European Secretariat of National BioIndustry Associations: its major role in Brussels is to channel industrial views into the European Community legislative mechanism.

All this indicates the presence of Assobiotec within a consistent network of interactions, at both national and international level with members which are carefully represented, individually and collectively.

Supporting Commercial Interests of Associate Members

Table 1 provides a synthetic view of industrial indicators on new biotechnology in Italy; it can be observed that R&D investments are still exceeding sales and that the number of companies is quite limited. Within this context—which has not substantially changed in recent years—Assobiotec promotes and executes the following:

—Editing a directory of the association, which provides member company profiles: the commitment in biotech-derived products of each company is given with indications on the specific technologies used and/or developed, their fields of application and, when appropriate, current products and projects.

—Reporting detailed members' profiles and activities in the Association Newsletter.

—Sponsoring conferences and exhibitions relevant to industrial development of biotechnologies.

—Channelling information and organizing meetings on international technology transfer opportunities.

Table 1. A Synthetic View of Industrial Indicators on Biotechnology in Italy

Sales (1990)	$250 Million
R&D investments (1989)	$350 Million
Number of companies	150
Number of employees	7,000

Source: "Il Progresso delle Biotecnologie in Italia"—Assobiotec 1990

These activities are coherent with the policy orientation of providing a regular forum for the exchange of information and initiatives of entrepreneurial relevance to members: business opportunities at international level are very often channelled through the association's services/initiatives.

However, Assobiotec does not have a professional role in business-related services (financing, technology transfer, strategic partnerships/alliances, ...).

Providing Updated Information

Assobiotec is provided with online access to major bibliographic-type commercial databases through PSDNs from international hosts such as Data-Star, BRS-Maxwell Online and Dialog. Commissioned searches and compilations, tailored reports and scientific/technical bibliographic selections are currently available.

Information activities on a regular basis include:

—Editing of synthetic and periodically updated monographic reports (with diffusion restricted to members) giving an outline of recent developments and perspectives of biotech-derived products and/or processes in a specific area, particularly those close to industrialisation (i.e., excluding basic research). At present 14 titles are available.

—Periodically editing the abstracts of the patents filed with the European Patent Office in genetic engineering, together with an analytical index which allows the retrieval of the patents by subject.

—Editing, within a collaboration agreement with the National Research Council (CNR), of reports providing summary of objectives/results related to public-funded biotech R&D.

—Publishing a newsletter, a periodical leaflet providing biotech news updates, national and international regulatory initiatives, information from members, the association's activities, conferences diary, etc.

The association's information and viewpoints are currently available on request (e.g., media, politicians, officials, pressure groups, students, etc.); they are regularly and openly expressed by board and staff members in books, articles, meetings, interviews etc. In particular, an Assobiotec "press release" is currently published in BIOTEC, the Italian magazine devoted to biotechnology. In 1990, the association edited a book (in Italian) on the Progress of Biotechnology in Italy.

Assessing Manpower, Education and Training Needs

Assobiotec is formally in charge of up-to-date evaluation of industrial needs of skilled workers/professionals, at both national (i.e., MURST—Ministry of University and Research) and international (i.e., BEMET—Biotechnology in Europe: Manpower Education and Training) level. An association representative is member of the National Committee for higher education in biotechnology.

Several projects in this area have been managed and implemented:

—a UETP (University Enterprise Training Partnership) with Northern Italian universities within the COMETT (Program On Cooperation between Universities and Industry regarding Training in the Field of Technology);

—training courses for high school teachers;

—short monographic courses.

The association is currently supporting students' doctorate thesis and channelling manpower offers/requests.

Conclusions

Regulation, intellectual property protection, biosafety and harmonization of procedures are the main areas of interest of Assobiotec; several working groups are currently involved in those subjects.

Board and staff members are either members or experts in several national commissions dealing with the safe use of new biotechnology in Italy, set up by:

—the Ministry for Community Policies, within the Prime Minister's Authority;

—the Ministry of Health (contained use of Genetically Modified Organisms (GMOs));

—the Ministry of Agriculture and Forestry (deliberate release of GMOs).

Furthermore, an Assobiotec staff member has been appointed by MURST as one of the Italian representatives at the OECD's "Group of National Experts on Safety in Biotechnology." Assobiotec is currently chairing the sub-commission "Biotechnology" of the Ente Nazionale Italiano di Unificazione (UNI) and is the UNI spokesman at the CEN—Technical Committee 233 "Biotechnology." At international level, Assobiotec is strongly supporting the activities of ESNBA activities in the area of "patents and regulations."

Other activities—such as information, training and business fora—are well developed but are mainly representing the coherent completion of a major political role at national and EEC level.

Training and Education

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[Article by Gian Tommaso Scarascia Mugnozza and Enrico Porceddu, Universita della Tuscia, Italy: "Italian Education and Training in Biotechnology"]

[Text] Biotechnology can be described as the utilization of living organisms in the production of goods and services. This definition also covers traditional agricultural and industrial activities as well as the more recent sectors of antibiotics and proteolytic enzymes. Over the past few years, man's ability to carry out carefully aimed and designed interventions into biological processes has increased, as has his ability to widen the field of application of these processes. Recombinant DNA technology, allowing for *in vitro* manipulation of genetic information from living organisms; cell culture technology; knowledge of chemical-physical and catalytic properties of biological macromolecules, allowing for the construction of new functions and properties; reaction and process control technologies and new purification and separation methods are only a few of the many possibilities that have become available to the world of health, industry, agriculture and environmental safety.

Despite all the studies and research undertaken in the various sectors of advanced biotechnology, it was not until the end of the 1970s that its potentiality and possible impact (also economic) in these sectors was realized. In the wake of events in the U.S., industries from the major industrialized countries, such as Great Britain, Germany, Holland, France, and the European Economic Community have taken their own initiative and encouraged highly coordinated government programs directed towards determining development in the sector.

The first training courses in biotechnology according to the different industrial interests and the educational systems, obviously varied from country to country.

In Germany, advanced training in this sector was primarily focused on the specialization acquired in the preparation of a Ph.D. thesis, while technician training courses were set up in collaboration with important institutions. Advanced training was therefore extremely specialized. The national program of applied biology and biotechnology, developed between 1983 and 1988, foresaw as one of its objectives, the setting up of fellowships for graduate specialization by means of multidisciplinary

research activities. A biotechnology course was established in 1987 in Lower Saxony and was then followed by courses in the Universities of Braunschweig and Berlin.

In Great Britain, the autonomy enjoyed by universities in the formulation of teaching programs has led to curricula made to suit the student and the local economic situation. The different levels of Bachelor, Master and Doctorate have all been developed following recommendations in the Spinks Report of 1980 and the development of scientific parks. The experience gained by the Netherlands in this field is of particular interest. Leiden University and Delft Polytechnic formed a partnership in order to set up the Delft-Leiden Biotechnology Center (BDL) which is directed towards retraining industrial personnel. The BDL has proposed a complete program, divided into three levels: the first, for a period of four years, provides biotechnological training together with a deeper knowledge of one or two other disciplines. The first level is followed by a common two-year course, leading to the attainment of a multidisciplinary diploma. The third level is represented by the doctorate.

Delft University also offers various post-university courses, ranging from microbe physiology and fermentation technologies to downstream control processes and the development of "business" technology. A degree course in biochemistry and biotechnology has finally been set up in the Faculty of Medicine at Amsterdam University.

France's approach has been remarkably different from the others. The university system has established first and second level degree courses in the various biotechnological disciplines. A number of post-graduate specialization and revision courses exist in the universities, the Pasteur Institute, high schools, national schools and national institutes of agricultural science.

The situation in Italy, is in an evolving stage.

Activities started with the institution of a degree (MS) course in agro-industrial biotechnology at the Faculties of Mathematical, Physical and Natural Sciences at the University of Verona; they are now progressing in a framework divided into three levels.

The following didactic structures herein reported are ready to come into action as a consequence of the almost completed legislative procedure.

The 1991-93 plan foresees:

1. The transformation of the so-called "special purpose schools" [Scuole a fini speciali] into (Dc) University diploma courses (Table 1).

Table 1. The Transformation of the So-Called "Scuole a fini speciali" Into University Diploma Courses

Technician in Medical Biotechnology
—Biotechnology
—Medical biotechnology
—Biomedical sciences orientated towards biotechnology
Biotechnology
—Biotechnology
—Chemical biotechnology
—Biotechnologist
Agro-industrial Biotechnology

2. Setting up of MS courses

—Agro-industrial biotechnology at the University of Aquila (Avezzano).

—Biotechnology, in the Faculties of Mathematical, Physical and Natural Sciences, Industrial chemistry and pharmacy at the Universities of Catania, Cosenza, La Sapienza—Rome (decentralized seat of Latina), Tor Vergata—Rome, Milan, Pavia (decentralized seat of Mantua), Perugia, Padua, Camerino, Urbino, Turin.

—At the University of Milan, the first two introductory years of the biotechnology course take place in the Faculty of MPN Sciences while the following years are divided between the Faculties of Pharmacy, Medicine and Surgery and MPN Sciences.

3. Setting up of Biotechnology MS courses at the Universities of Bologna (jointly between faculties of MPN Sciences, Medicine and Surgery, Pharmacy and Agriculture), Genova, Naples—Federiciana, Rome—La Sapienza and Turin (jointly Medicine and Surgery, Pharmacy and MPN Sciences).

4. The research doctorates were composed of only "Food biotechnology" courses in the first, second and third cycles, in the Faculty of Agriculture at the Universities of Milan or Bologna. After the fourth cycle the number of doctorates increased to eight, one of which was in biotechnology applied to pharmacology and cellular and molecular biotechnology applied to the pharmaceutical sector in the Faculty of Pharmacy at the University of Milan. The other doctorates include fungi biotechnology at the University of Perugia, cellular and molecular biotechnology applied to the biomedical sector in the Faculty of Medicine and Surgery at the University of Brescia, biotechnology and biomedtrics at the University of Aquila and molecular biotechnology in the Faculty of Udine at the University of Milan and finally food biotechnology in the Faculty of Agriculture at the University of Udine.

In January 1992 a commission was set up with the task, among others, of scrutinizing new possibilities at different faculties and universities.

The commission proposed the setting up of five degree MS courses having different professional objectives: industrial, medical, veterinary, pharmaceutical and agricultural biotechnology.

The commission also proposed the appointment of a Biotechnology Committee at each university. Estimating the resources this committee should indicate, on a rational basis, the activation of one or more courses (if two or more are activated then they should have at least one course in common). Alongside the Agro-Industrial Biotechnology degree courses authorized for Verona and Aquila, another five courses should consequently be established. The disciplines that characterize each course indicates the multidisciplinary character of the courses, especially those belonging to the introductory part. The strong interdisciplinary connotation of these courses is the opportune counterpoint that makes biotechnology a field of great scientific and technological convergence.

The hour glass provides a very accurate example of this situation, representing biotechnology in the center, then subdividing and diversifying into the relative applicative sectors. This comparison is not accidental; the focal point of biotechnology lies in its interdisciplinarity. The degree course is completely different from any other attainable from the faculties in which it taught, with one exception—agriculture which has always been characterized by its interdisciplinary features and for this reason has been under strong criticism. A great obligation faces the teaching staff due to the fact that the single disciplines constituting the courses have been manipulated and geared towards a single objective. In some ways this limits the freedom of the teachers, as far as the legislators are concerned this freedom has been too idealogical and orientated towards methods rather than scientific content. Despite this the knowledge that can be derived from the different sectors, the interlacing between fundamental chemistry, physics and biology must surely create a professional in control of highly innovative tools necessary to face and resolve practical problems specific to the different sectors who can collaborate with the traditional medical doctors, veterinarians, surgeons, pharmacologists and agronomists, given that he is. Therefore, the focal point of the different courses does not exist in the presence of common disciplines such as mathematics, physics, chemistry or biology etc., because they constitute a part of each element, as much in the basic teaching of them in a situation in which their knowledge is integrated within a system.

Disciplines are not purely considered as an introduction, but they have as a final goal the attainment of a sensitivity that makes them at the same time the tools and the means of their learning and utilization. This can be obtained when teachers of these disciplines take an active part in all motivities of a faculty, including research. In this way a true and deep modernization will take rediscovering the true spirit of a university: to teach at the frontier of science and technology.

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